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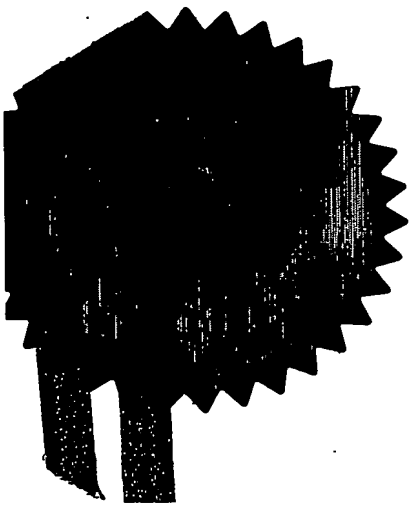

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Dated 17 September 2003

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0219248.2

2. Patent application ...
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17 AUG 2002

19AUG02 E741973-2 D10007
P01/7700 0.00-0219248.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

UNIVERSITY OF YORK
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Patents ADP number *(if you know it)*

04169546001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

OPTICAL ASSEMBLY AND METHOD FOR DETECTION OF LIGHT TRANSMISSION

5. Name of your agent *(if you have one)*

NOVAGRAAF PATENTS LIMITED

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

THE CRESCENT
54 BLOSSOM STREET
YORK YO14 1AP

Patents ADP number *(if you know it)*

07296486002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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b) there is an inventor who is not named as an applicant, or
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Claim *(s)*	hw
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11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 16/08/2002

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12. Name and daytime telephone number of person to contact in the United Kingdom PHILIPPA M ALLEN 01904 610586

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OPTICAL ASSEMBLY AND METHOD FOR DETECTION OF LIGHT TRANSMISSION

The present invention relates to an optical assembly comprising a sample vessel positioned in a direct light path between a light source and a light detector, in manner to enable transmission of light through the vessel; a method for detection of light transmission through sample contained within the vessel; an apparatus comprising the assembly; more particularly an apparatus for sample analysis for example for high throughput screening (HTS) or profiling or assays, such as enzyme assays; and uses thereof in the pharmaceutical, biomedical and bioscience, agrochemical, veterinary, materials and like fields, for detection, analysis, characterisation and quantification or the like of samples contained in a vessel, and optionally further collecting separated components thereof; in particular in combinatorial chemistry; in metabolomics, proteomics or genomics, assay and high throughput analysis applications, typically high sensitivity analyses, separation and/or quantification studies and for sample separation for example chromatography or electrophoresis, in particular column chromatography, capillary electrophoresis with real time or post separation analysis.

UV absorbance, fluorescence and mass spectrometry are key technologies used in separation science for analysing species in samples. A particularly useful methodology is to look at a sample population separated by capillary electrophoresis with fluorophore labelling and fluorescence imaging, for quantification, and MS for characterising molecules of interest.

US 5,582,705 discloses an apparatus and system for laser induced fluorescence (LIF) detection in a multiplexed capillary electrophoresis system. A coherent beam incident on the capillary array and emitted fluorescent light

are typically perpendicular to each other in order to reduce background noise due to light scattering. A transparent portion in each capillary wall defines a transparent path extending through the array, perpendicular to the capillary. A 2D image array detector such as a charge-coupled device (CCD), preferably a charge-injection device (CID), is positioned to detect emission, and an imaging lens interposed between the capillary array and the image array detector, to optically couple the pixels to the capillary. The imaging lens may be any lens capable of transforming an image onto the pixels of the image array detector, such as a camera lens or a condenser lens. Coupling is shown in Figure 4, of US 5,582,705 in which every second pixel is coupled to a sidewall of the capillary and every pixel in between is coupled to an interior portion.

Fluorescence detection is limited in its application since only a limited number of molecules are naturally fluorescent and many have to be derivatised in reproducible and quantitative manner. Absorbance detection therefore has the advantage of enabling detection of a wider range of molecules. For example in enzyme assays, conducted in microtitre wells, techniques can be extended to absorbance detection of chromophoric, UV and vis absorbing substrates consumed or produced in an assay, extending the range of assay to natural as well as synthetic substrates.

However a fundamental limitation of the use of single point absorbance detection is the impossibility of creating an image of the source at the detection point on capillary that is brighter than the light source. In "A charge coupled device array detector for single-wavelength and multi-wavelength ultraviolet absorbance in capillary electrophoresis", Bergstrom and Goodall, Pokric and Allinson, Anal. Chem. 1999, 71, 4376-4384 discloses optical detection in capillary electrophoresis by means of absorbance detection,

illuminating a length of the capillary using a fibre optic bundle and using a charge coupled device (CCD) camera to image the full length of the illuminated zone. In this publication light from a fibre optic bundle is focused by a sapphire rod through the capillary core and detected on the opposite side of the capillary, by this means, increasing the target light area enabling more of the lamp output to be used and increasing the total light flux. In this case light emanates from the capillary core, so all light detected is useful and the divergent beam obtained is imaged on to the CCD.

Such a system becomes more complex once a parallel capillary array is introduced in place of the single capillary. Optics to focus light on the core of each capillary would be extremely complex and therefore irradiating both the core and walls of each capillary becomes a practical consequence.

WO 01/18528 (Yeung et al) discloses a method for analysing multiple samples simultaneously by absorption detection of samples in a planar array of multiple containers, whereby stray light from adjacent containers is eliminated by distancing the detection means from the array, preferably at a distance greater than 10 times the diameter of a container, suitable 10-100 times the diameter for example at a distance of 1-30 cm. Containers are preferably cylindrical capillary tubes as shown in the art. The array comprises a control container if the light source is unstable. It is stated that the cross section of the container and thickness of the capillary wall are not critical. A flat field lens preferably images the containers on to the detection means.

We have now found that further improvements in absorbance detection assemblies enables simplification of optical components, without unduly large separation of capillary and detector which is undesirable and reduces light collection efficiency compromising path length, and therefore light intensity.

The improved assembly is of particular advantage in detection in multiplexed capillary arrays and enables imaging a large area of a capillary array without the need for imaging optics. This is a significant advantage, especially when working in UV where it is very difficult and expensive to produce suitable optics. The assembly has benefits however in both single capillary and array detection, in particular enabling a simple and improved exposure referencing and acceptably low intercapillary cross talk without the need for optics.

Accordingly there is provided in the broadest aspect of the invention an optical assembly comprising a light source, at least one sample vessel and a detector, the at least one vessel being positioned in a light path created between the source and detector in manner to enable transmission of light through the vessel wherein the light source is adapted to provide a beam of substantially collimated light, the detector comprises a plurality of detector locations and the vessel comprises a wall and core of relative shape and dimensions adapted to define at least two spatially separated transmitted light paths, a first wall path which enters and exits the vessel walls only, spatially separated from a second core path which enters and exits the vessel walls and additionally the vessel core, wherein the spatially separated wall and core paths are coupled to individual detector locations on the detector. Preferably the detector is an array detector. Preferably the detector is adapted to detect and provide information on respective wall and core path light transmission. Preferably the assembly is coupled to means for displaying information on respective wall and core path light transmission or for displaying referenced information on core path light transmission, referenced against wall path light transmission.

Preferably the assembly defines a central core path and two peripheral wall paths either side thereof or an annular wall path thereabout.

In a particular advantage, the apparatus of the invention enables exposure referencing of a light beam traversing a core path of the at least one sample vessel, by a light beam traversing a wall path of the same sample vessel. The beams are spatially close, preferably adjacent, on the array detector, facilitating direct referencing as the ratio of the core beam to the wall beam. In a further advantage the two light beams are of neighbouring origin whereby core and wall beams have a high probability of emanating from the same region in the light source eliminating the effects of light source fluctuations due to e.g. instability or spatial inhomogeneity. The assembly of the invention is therefore able to operate at the shot noise limit.

Preferably the light source comprises at least one wavelength of light that is absorbed by one or more absorbing species, the absorption of which is to be detected. More preferably the light source comprises a deuterium, iodine, zinc, cadmium or mercury lamp, more preferably an arc lamp (for UV light absorption); or comprises a xenon lamp, more preferably an arc lamp (for UV-vis light absorption); or comprises a tungsten lamp, more preferably a filament lamp (for visible light absorption), and the like; most preferably a high output arc lamp selected from the above. Alternatively the light source can be a laser, for example in the UV a laser such as a frequency quadrupled Nd:YAG at 266 nm or Nd:YLF at 262 nm, or a He-Cd laser at 325 nm. Wavelength selection in the case of continuous wavelength arc lamps for example, is suitably by known technique such as interference filter positioned between the light source and collimating means or between the collimating means and the sample vessel, preferably before the collimating means. An optical filter may be in the form of a monochromator such as a grating and this is continuously variable, alternatively a filter wheel or variable interference filter may be employed for sequential wavelength detection. Light may be of wavelength in the range 180 to 1200 nm, corresponding to UV-vis to near

infra red (NIR), and is preferably in the range 180 to 700 nm corresponding to UV-vis. The light source output may be coupled into a fibre optic if desired for remote or zone illumination. Coupling the output into a single optical fibre reduces noise contributions caused by fluctuations in the spatial distribution of the lamp discharge.

The light source may be expanded and recollimated by known means, for example using cylindrical and spherical lens elements and the like, preferably using an elongate lens or cylindrical optical component, such as a cylindrical fused silica lens or the like, to produce a collimated beam suitable for zone illumination of a sample vessel array.

The at least one sample vessel in the assembly of the invention may comprise a cell or conduit which may be closed or open ended and closed or open based and topped, intended for static or dynamic sample detection. The vessel may be aligned for light transmission in any suitable plane through the vessel. Suitably light transmission is through a plane perpendicular to or containing the vessel ends or base and top.

Preferably the sample vessel is a single cell or one of a plurality of cells in an array, such as a rectangular or square array, for example in a microtitre plate, well plate or multi sample plate; or is a capillary such as a microcapillary or microfabricated channel as known in the art of microfluidic transport and separation, more preferably is a single capillary or microchannel or one of a plurality of capillaries or microchannels in a parallel array.

A sample vessel array is aligned in a plane perpendicular to the collimated light path whereby light passes one vessel only. In the case that direction of illumination is in a plane containing the vessel base and top, light enters and

exits each vessel through a sidewall (wall path) or enters through the top and exits through the base (core path), and in this case vessels may be aligned in a parallel or matrix array; in the case that illumination is through a plane perpendicular to the vessel ends, light enters and exits each vessel through a near side sidewall (wall path) or enters and exits through a nearside sidewall, emerges into the core, enters and exits an opposing sidewall (core path), and in this case vessels may be aligned in a parallel array, ie only one vessel deep.

For 1:1 illumination:detection, the collimated light path has dimensions substantially corresponding to the width of the array and to a desired length of each sample vessel which it is desired to optically detect.

Light passing through the vessel walls and core is refracted on entering and exiting the wall(s) and additionally on entering and/or exiting the core. In the case of illumination through the length of a cell or capillary, or through the cross-section of a straight walled cell or capillary, emergent wall and core light paths maintain their respective orders, ie there is substantially no cross-over or convergence of respective paths, at least within a distance d to the detector.

In a particular embodiment of the invention the vessel of the assembly is a microfabricated device providing square cross section capillaries, and is suitable for Fabry-Perot illumination with enhanced light absorption through multiple passages through the vessel. In this case the walls of the vessel are coated with a reflective coating, within an absorption zone, whereby light enters the vessel core in an illumination zone adjacent the absorption zone, through a nearside side wall, at an angle less than 90 degrees to the wall, and at least a portion of light is reflected at the opposing wall, with repeated internal reflections throughout the absorption zone and finally emergence from

the opposing wall at the end of the absorption zone. Light traversing a wall path may be similarly reflected for ease of alignment but is preferably not reflected and exposure referencing is performed as normal.

In the case of illumination through the cross-section of a curved walled cell or capillary, for example a circular cross-section capillary, emergent wall light paths reverse their respective orders about a central core light path, ie there is cross-over of respective wall paths, within a distance d to the detector. In this case the optical assembly of the invention is characterised by refraction patterns through the vessel in order to be able to spatially separate wall and core light paths. Preferably the assembly is characterised by respective outer and inner diameter of a sample vessel, whereby the wall and core paths are spatially separated as hereinbefore defined.

Preferably the vessel outer wall and inner wall are of shape and dimension whereby light transmitted through the core is convergent and forms a beam having an undeflected beam path, i.e. having a beam path continuous with the incident collimated light. Light passing through the wall of the sample vessel only, entering the wall at one outside wall location and exiting at a second outside wall location may be deflected or undeflected, preferably if deflected is divergent with respect to the core light path such that two classes of light paths are formed which are spatially separated.

It will be appreciated that by virtue of using a collimated light source and knowing the refractive index of the sample vessel and any sample contained therein the light path passing through any point of the vessel can be predicted for any given shape and dimension of vessel outer wall and inner wall, chosen such that collimated light passing through a square or rectangular cross section vessel, normal and parallel to respective walls, emerges substantially

unrefracted and parallel; and collimated light passing through a curved or angular cross section vessel emerges uniformly divergent or convergent with a gradation in angle of refraction. This enables production of an emergent light path of high symmetry and/or uniformity, which can be manipulated for imaging and exposure referencing as hereinbefore defined.

Preferably the at least one sample vessel has a cross-section in a plane including the transmission light path, which is square or rectangular, curved circular or angular or a combination thereof and is symmetrical or asymmetrical, preferably is symmetrical. The sample vessel moreover comprises an outer and inner wall which may be of similar cross-section or shape or may be different, for example one may be circular and one square. The sample vessel wall through the cross-section may be continuous or non-continuous, for example the vessel may be open or closed and is preferably closed. A closed vessel may have a continuous wall through its cross-section or may comprise continuous base and side walls with a separate seal or lid. Preferably the outer wall is square open or closed or circular closed, and the inner wall is square or well shaped open or closed or is essentially square with microfabricated convex or concave inner wall sections acting as lens faces, or concave, convex or prism shaped outer wall sections acting as lens faces.

Preferably at least one of the outer and inner walls of the sample vessel is of circular cross-section, whereby refraction and spatial separation of core and wall beams is achieved, preferably outer and inner walls are of coaxial circular cross-section thereby defining an annular wall having outer and inner diameters such that refraction and spatial separation of core and wall beams is achieved.

It will be appreciated that sample vessel dimensions may be selected according to the nature of vessel wall shape and sample to be contained within the core in order to achieve the desired refraction and spatial separation. Preferably therefore for example a sample having circular outer and inner wall cross-section and a vessel to detector separation equal to the outer diameter (o.d.), the o.d. and inner diameter (i.d.) of the annular wall of a sample vessel are defined by the relation: i.d./o.d. =A, wherein A is in the range greater than or equal to 1/4, preferably in the range 1/4 to 5/6, more preferably in the range 1/4 to 2/3, most preferably in the range 1/4 to 1/2. Suitably the sample comprised within the vessel has a refractive index in the range of 1.30 to 1.40 for example 1.33. Results for different samples are shown in Table 1 below.

The vessel may be associated with additional optical components in the emergent light path, to focus or otherwise manipulate one or both of the spatially separated beam types.

The array may comprise fixed or variable spacers between each sample vessel for adjusting spacing of emergent beams B in a sequence for vessels 1 2 3 etc, as shown in Figure A, having wall w and core c, for example wherein each beam corresponds to an array detection location:

B1w B1c B1w B2w B2c B2w B3w B3c B3w...

If desired, adjacent wall beams may be coincident:

B1w B1c B1w/B2w B2c B2w/B3w B3c B3w/...

Other out of step arrangements are possible but may require more complex coupling and reading of detector location.

Spacers may comprise elongate filters designed to screen all or part of a wall light path, or a filter to screen part of the vessel wall thereby confining the wall light paths, in both cases for minimising intercapillary stray light and cross talk.

A spacer may therefore be of any suitable material for combined spacing and filtration, optionally also serving as support means for the at least one sample vessel, and is of suitable opaque material such as for example an opaque glass filled polymer.

Sample vessels may be comprised of any transparent polymer, glass, quartz, silica, e.g. fused silica or other material, preferably optical grade, more preferably having low levels of fluorescence. Optical grade polymers include the classes of siloxanes such as polydimethylsiloxane (PDMS), amorphous polycycloolefins based on norbornene (Nippon ZEON Corp), and polymethylmethacrylate (PMMA, DQ501, Rohm GmbH) which has low levels of fluorescence, and the like.

The vessel wall may be transparent in part or whole and is preferably of the same material throughout.

A capillary as hereinbefore defined may comprise any extruded capillary or microfabricated capillary or channel or other parallel elongate closed conduit means. A capillary may be flexible or rigid and may be straight or curved along all or part of its length.

Capillary arrays and microfabricated channel arrays are commercially available. Capillary arrays are typically manufactured by drawing or extrusion, creating a void circular cross-section core of high uniformity dimensions. Microfabricated arrays are typically manufactured by injection moulding from a tool, creating a void square or rectangular cross-section channel, which is then optionally sealed with a cover layer, and offers the advantage of absolute reproducibility of highly precise channels, and freedom of channel design.

A sample vessel may comprise part of an array of any number of vessels, commercially available arrays include 8 to 1536 vessels, preferably 8 to 520 capillaries or 96 to 1536 wells, for example 8, 12, 16, 24, 32, 48 or 96 capillaries or 96, 384 or 1536 wells.

The vessel may comprise part of an array of a greater number of vessels which may be available in the future.

Micro fabricated arrays fabricated in layered planar manner, constructing interfacing elements of a device in sequential planes, are gaining acceptance and widespread use. They have numerous advantages in accuracy, reproducibility, ease and flexibility of manufacture.

Vessels may have any desired separation, suitably are fused or adjacent or are spaced apart. A lower separation increases packing density but a higher separation reduces intervessel interference, in particular optical interference. It is a particular advantage of the invention that intervessel spacing may be used. This is not possible in the embodiment of Yeung *et al* above, for example which needs minimal vessel separation to minimise stray light.

The vessel may be of suitable length and depth according to the desired use. Preferably for capillaries or channels length is in the range 2 cm - 2 m for example in the range 2.5 – 6 cm for microfabricated channels or 6 - 100 cm length for capillaries, more preferably 10 to 60 cm. Greater length allows application of higher voltage in capillary electrophoresis and improved separation. For microtitre plate well, preferable inter-vessel centre to centre spacings are compatible with standard microtitre plate formats, such as 2.25, 4.5 and 9 mm.

Preferably a vessel is of 3-380 micron in depth, internal or external diameter, more preferably less than or equal to 200 micron.

Preferably sample vessel is of internal diameter (hereinafter i.d.) in the range 20 to 250 micron, preferably 20, 25, 50, 75, 100 or 250 micron, and of external diameter (hereinafter o.d.) preferably in the range 100 to 380 micron, for example 150, 200 or 360 micron. For example a vessel may be of 75–100 micron i.d. and 194 micron o.d. or 180 micron i.d. and 364 micron o.d. A vessel wall is importantly of suitable thickness for the optical properties of the invention. Thickness is suitably in the range 30 - 150 micron, vessels of low i.d. preferably have low wall thickness, to give a minimum o.d. to i.d. ratio of 2 - 4.

The sample vessel may be void or comprise stationary phase or may be coated or otherwise configured with suitable materials as known in the art for example in HPLC.

The separation between a sample vessel and the array detector, d, is suitably such as to facilitate coupling of spatially separated light paths to the detector

locations. Suitably the separation is a function of the vessel o.d. and of the degree of separation of light paths, and is preferably given by the ratio d: o.d. is less than 10:1, for example is 5:1 – 0.5:1, more preferably 1:1 – 0.5:1. In a particular advantage the optical assembly of the invention allows an extremely compact structure in which separation d is of the order of 50 – 360 micron, for example 200 micron. This is particularly advantageous since the lesser the value d the more compact and robust is the assembly and the more intense are the transmitted light beams at the detector. The separation d may be adapted to couple spatially separated light paths in sequence as hereinbefore defined, with or without intervening optical components and preferably without intervening optical components.

There are a number of commercially available array detecting systems, including for example the commonly used photodiodes and more recently available charge coupled devices (CCDs) and active pixel sensors (APSs).

Preferably therefore an array detector according to the invention comprises a solid state sensing device, more preferably a CCD, CID or APS.

A detection zone may be of any desired area size and is suitably of zone dimensions substantially equal to the vessel or array cross section in a plane perpendicular to the light path, corresponding to one to one image. A detection zone may comprise one or a plurality of array detectors.

Smaller zone size increases noise and reduces sensitivity yet can be provided at lower cost. Preferably the apparatus comprises lowest noise and highest

sensitivity CCDs in a relatively small area device, such as a 1024 x 256 pixel CCD (MAT CCD30-11).

A CCD for use in the apparatus of the invention may have 22 to 5000 or more pixels in either dimension, preferably 256, 512, 770, 1152, 2048 or 4096 pixels, of pixel size 7 to 35 micron, preferably 20 to 30 micron, more preferably 22 to 30 micron. Preferably the CCD comprises 3 to 28, for example 10 pixels per capillary or 30 to 2500 for example 100 pixels per well. Pixels outside the imaging area are preferably not digitised to reduce readout time.

Commercially available CCDs may include a stud, for protection of the CCD surface which is usually recessed in the CCD support package and to conserve image quality. Preferably the stud comprises a coating to absorb incident light and reemit at a different wavelength, to convert UV to visible light, to allow detection by the CCD. A coating is for example a phosphor coating. The phosphor coating may be applied directly to the stud or to a cover slip interleaved between the stud and capillary, facilitating changing phosphor as needed.

Each camera is interfaced to control means, preferably a processing control means providing a suitable pixel readout rate, suitably of the order of MHz, preferably greater than 4 or 5 MHz.

Preferably the processing means is programmed for selection of on-chip charge binning procedures, to increase signal current (photoelectrons per second) and selection of area of interest for read out. Additionally the

processing means controls camera readout and collects, stores and analyses data.

In the case of an assembly comprising a conduit sample vessel intended for dynamic sample detection, preferably the apparatus operates with real-time signal processing for optimum peak detection and parameterisation/characterisation, and potential for automatic system management including closed-loop feedback control of the apparatus and systems. Closed loop feedback may include controlling velocity of samples in multiple vessels, for example in a capillary array, to co-ordinate exit times, in sequence or, for combining samples from different capillaries into a common exit means, simultaneously. Controlled exit may be for subsequent fraction collection or analysis, for example for interfacing the output of a capillary bundle to a mass spectrometer by electro-spray or the like.

In a further aspect of the invention there is provided a method for detection of light transmitted through at least one sample vessel as hereinbefore defined containing a light transparent sample within its core, comprising illuminating the vessel with a substantially collimated light source and detecting transmitted light in an array detector, wherein transmitted light is spatially separated into at least two light paths, a wall path which has passed through the vessel walls only, spatially separated from a core path which has passed through the walls and core, wherein the spatially separated light beams are coupled to individual detection locations on the array detector.

Preferably the method is a method for detecting light from samples in sample analysis for example for high throughput screening (HTS) or profiling or assays, such as enzyme assays; and uses thereof in the pharmaceutical,

biomedical and bioscience, agrochemical, veterinary, materials and like fields, for detection, analysis, characterisation and quantification or the like of samples contained in a vessel, and optionally further collecting separated components thereof; in particular in combinatorial chemistry; in metabolomics, proteomics or genomics, assay and high throughput analysis applications, typically high sensitivity analyses, separation and/or quantification studies and for sample separation for example chromatography or electrophoresis, in particular column chromatography, capillary electrophoresis with real time or post separation analysis.

The method may be a method for detecting light from a static or dynamic sample. Typically a static sample is contained in a closed ended sample vessel as hereinbefore defined, more typically in a vessel in the form of a cell or well which may be one of a plurality of vessels for example in a microtitre plate or a well plate or sample plate as hereinbefore defined. Samples derived from enzyme assay or other multiple sample analysis may be in this form for detection.

Typically dynamic samples are electrically or pressure driven and are present in an open ended conduit type vessel as hereinbefore defined, such as a capillary having inlet and outlet ends for flow of sample through the capillary, the capillary may be a single capillary or part of a capillary array as hereinbefore defined. Samples derived from high throughput screening or from the separation or transport techniques may be provided in this manner for detection, of which samples derived from separation techniques may be provided for separation within the capillary with simultaneous detection of light transmission, or may be separated in a separation method such as column chromatography, and the separated sample flow from the column coupled directly into a capillary for detection of light transmission.

Capillary separations known in the art are suitably selected from pressure driven or electrical driven separations including HPLC (for pressure driven) or capillary electrochromatography (CEC) (electrically driven equivalent of HPLC, separating by binding or partition coefficient, using eg hydrophobic stationary phases), micellar electrokinetic chromatography, and capillary electrophoresis (CE) including the focusing and concentrating techniques of isoelectric focusing (IEF separating by isoelectric point independent of size and shape of molecules), isotachophoresis (ITP) and capillary zone electrophoresis (CZE separating by charge to size ratio). Other electrically driven techniques are known or may be developed in future.

In these processes electrophoretic separation of molecules is carried out in a capillary or channel which is connected to a buffer supply. An electric field in the range of kilovolts is applied across both ends of the capillary or channel to cause the molecules to migrate. Samples are typically introduced at a high potential end and, under the influence of the electric field, move toward a low potential end of the channel.

The method for detection of light from a plurality of sample vessels may therefore be a method for high throughput analysis of different samples in each vessel, for example for comparative analysis thereof or may be a high loading method for detecting the same samples in each vessel, intended for combining a desired component from each sample. High loading in this manner achieves higher sensitivity in detection and higher resolution in separation than operation on a large scale from a single large bore separation channel.

Preferably the method comprises illuminating the at least one sample vessel with collimated light comprising a single or a plurality of wavelengths selected in the range as hereinbefore defined. In a particular advantage illumination is with a collimated light source of a single wavelength at any given time, thereby simplifying readout of optical detection results.

A sample as hereinbefore defined may comprise any sample of one or more small or large molecules present in liquid phase suitably in solution with liquid phase solvent or cosolvent such as an inert or non reactive liquid. Suitably however these samples are characterised by a refractive index of similar order to that of the sample vessel walls, whereby the sample provides a generally convergent light path. Preferably therefore the sample comprises a solution or a suspension of molecules to be detected in a solution or suspending medium selected from water, alcohols, acetonitrile, acetone and other common solvents and cosolvents; or the sample may be provided in the form of a gel for example uncrosslinked polymer solutions such as cellulose derivatives (e.g. hydroxypropyl cellulose) and synthetic polymers (e.g. polyethylene oxide).

Preferably the method of the invention comprises selecting a sample for analysis, determining individual wavelengths at which absorption by desired sample components is strongest, checking refractive index of the sample in order to select a suitable sample vessel which when containing the sample and when illuminated will generate spatially separated beams as hereinbefore defined or selecting a suitable combination of optical components, filters and the like and a suitable vessel to detect an array separation to couple spatially separated beams to independent locations on the detector array.

Sample may be introduced into the at least one sample vessel as hereinbefore defined in known manner, for example by injection, loop injection, pipette, hydrostatic, electrokinetic or like injection techniques and may be removed from the vessel in known manner such as injection, electrospray or other interface for discard or to a further vessel for storage or to a down stream identification means such as mass spectrometer.

Detected light is coupled to individual detection locations on the array detector as hereinbefore defined. The at least one sample vessel as hereinbefore defined is coupled to a plurality of detector locations in manner that core detector locations correspond to the core light path from the at least one vessel and peripheral detector locations correspond to the peripheral wall point(s) from the vessel. Preferably the method comprises imaging the transmitted light detected by the detection means, for example in the form of a CCD image as known in the art. Preferably the method also comprises referencing the light detected by the detection means by means of exposure referencing wherein the ratio of the core beam intensity to the wall beam intensity gives a value for the sample intensity at each location with elimination of excess or flicker noise due to light source fluctuation.

Accordingly therefore the method comprises coupling the at least one sample vessel to at least three detector locations, preferably 3 to 25 detector locations for a sample vessel of the order of microns diameter wherein the locations may be apportioned for example 1:1:1 – 5:15:5 or 8:9:8 depending on the relative wall and core beam width; and for a sample vessel of the order of cm in an amount of 30 to 2500 pixels per sample vessel for example in the ratio 5:20:5 or 10:10:10 to 500:1500:500 or 800:900:800 depending on the relative wall and core beam width. The method may include pixel summing or the like for enhanced signal strength and noise reduction as known in the art.

Preferably the method includes subsequently measuring the amount of absorption of light by species in the sample vessel which indicates the amount of absorbing species in manner as known in the art and comprising measuring the intensity of light in the absence and presence of the sample. In a particular advantage measurement according to the invention is simply by measuring intensity of light in a wall beam and a core beam. The logarithm of the ratio provides the absorbance according to the Beer-Lambert Law.

Absorbance may be imaged, for example as a CCD snapshot. Preferably however the method is a method for snapshot detection whereby detection is recorded in a plurality of finite exposures, for example five exposures per second, and exposures are super imposed, directly in the case of a static example or with time displacement in the case of a dynamic sample. Accordingly an individual image reveals limited information and therefore the detection array preferably provides raw data which is compiled and converted to graphical display for example in the form of an electropherogram.

In a further aspect of the invention there is provided an apparatus for chemical synthesis and analysis or for sample separation or transport wherein the apparatus comprises the optical assembly as hereinbefore defined. The apparatus may comprise a plurality of means for introducing one or more reagents to the at least one sample vessel together with means for regulating reaction conditions to form a desired chemical synthetic reaction within the at least one sample vessel; or may include means for inlet and outlet of sample in dynamic fashion into the at least one sample vessel together with means for applying a potential difference across the ends of the vessel and means for supplying separation mediums such as buffer in order to perform separations within the at least one sample vessels: or may include a separation means such

as a chromatography column as known in the art having outlet interfaced with an inlet end of the at least one sample vessel as hereinbefore defined for dynamic analysis of separated sample.

In a further aspect of the invention there is provided the use of the optical assembly, method and apparatus as hereinbefore defined in sample analysis for example for high throughput screening (HTS) or profiling or assays, such as enzyme assays; and uses thereof in the pharmaceutical, biomedical and bioscience, agrochemical, veterinary, materials and like fields, for detection, analysis, characterisation and quantification or the like of samples contained in a vessel, and optionally further collecting separated components thereof; in particular in combinatorial chemistry; in metabolomics, proteomics or genomics, assay and high throughput analysis applications, typically high sensitivity analyses, separation and/or quantification studies and for sample separation for example chromatography or electrophoresis, in particular column chromatography, capillary electrophoresis with real time or post separation analysis.

The invention has particular use in relation to samples of small molecules of MW of the order 15 – 500, but may also be used in relation to samples of larger molecules such as polymers and biomolecules of MW of the order 500 to 10^6.

The invention is now illustrated in non limiting manner with respect to the following examples and figures wherein

Figure A shows a schematic of layout of a plurality of sample vessels of the invention.

Figure 1 shows a schematic diagram of apparatus of the invention.

Figure 2 shows the collimating means and detection zone of Figure 1.

Figure 3 shows the CCD and vessel arrangement of the optical assembly of the invention.

Figure 4 shows part of a CCD snapshot

Figures 5 and 7 show light beam tracings according to the invention.

Figures 6 and 8 show light beam tracings not according to the invention.

Figures 9-12 show electropherograms of microfluidic detections.

Example 1

The experimental setup is shown in figure 1. The output from a 75 W xenon lamp is launched into a single 1 mm diameter fused silica fibre. The light output from the fibre is shaped and collimated using cylindrical and spherical fused silica lens elements (figure 2) to illuminate the rectangular area of the CCD. The CCD chip used is (EEV CCD 30-11) and is thermostatted and controlled by a system designed and built by York Electronics Centre; it has 1024 by 256 active pixels, each 26 μm square, with a total active area of 6.7 x 26.6 mm. The CCD chip has a fibre optic stud (faceplate) that protects the CCD surface. The fibre optic stud is not UV transparent, so a UV phosphor is coated onto the surface of stud to making the detector sensitive to a wide wavelength range from NIR to below 200 nm. The charge accumulated on the CCD is read out in a series of snapshots, to prevent image smearing a light chopper ensures that the CCD is not illuminated during the readout period. An exposure rate of 5 Hz is used with a 50% duty cycle (100 ms exposure and 100 ms readout time). An image of 1024 × 256 pixels with 14 bit digitisation is obtained from each snapshot.

An ideal arrangement of the capillaries could be to align them parallel to the short axis of the CCD at a spacing of 260 μm (10 pixels per capillary). This

arrangement would allow up to 102 capillaries which is ideal to accommodated the number used if sampling from a standard 96 well plate. The spacing means a gap of 66 μm between each capillary, this would allow more reference light reaching the CCD than in the current arrangement with the capillaries more tightly packed. Better exposure referencing and lower inter capillary cross talk should result from this arrangement.

Capillary imaging

In the demonstration experiment four capillaries (100 μm i.d., 194 μm o.d. 500 mm long) were placed side by side, approximately 200 μm above the CCD and parallel to the long axis of the CCD as shown in figures 1 and 3. The portion of the capillaries imaged was a section 273.4 to 300.0 mm from the inlet end. Figure 4 shows a portion of one snapshot taken with the four capillaries filled with air, water and the last two with an ink solution. Excellent referencing is seen for the assembly without any lenses or other optics. It can be seen from the comparison between capillaries 1 and 2 that the collimated light that passes through the capillary core is convergent when the capillary is filled with water producing a line that is brighter than the background. Figures 5 and 6 show in detail how light passes through the water and air filled capillaries; they show also that in the case of the filled capillary, there is good separation on the CCD between the light passing through the capillary core and that only passing through the capillary walls. This is confirmed in figure 4 where a high contrast is observed between the images of the water and ink filled capillaries. It is important to use capillaries with a small o.d.; figure 7 shows that good results would be obtained using capillaries with the same o.d. of 194 μm as used here but with the more commonly used 75 μm i.d. However, the usual 364 μm o.d. capillary would not be suitable, figure 8 shows that the larger radius at the

air/fused-silica interface does not adequately focus the light having passed through the capillary core to spatially separate it from the light having passed only through the capillary walls. During an experiment the image from the whole CCD was read into the computer for each exposure; for this experiment an area of 32 × 1024 pixels contained the image of the four capillaries the remainder was discarded to conserve computer memory. The data was further reduced by adding together the pixel values in groups of four down the capillary length to give a total of 32 × 256 effective pixels for each snapshot; each effective pixel has 16 bit resolution and dimensions of 26 × 104 μm.

Exposure referencing

Fluctuations in exposure times and in the light source intensity that is in excess of shot noise need to be accounted for in order to get the best possible performance from the detector. This is usually achieved by using a double beam arrangement where a portion of the light from the source that does not pass through the sample is monitored and used as a reference. The ratio of this reference and the light that does pass through the sample is used to calculate the absorbance. In this experiment the light that strikes the CCD having passed only through the capillary walls is used as the reference. The electrophoresis of 4-nitrophenol in its ionic form was used to test the performance of the detection system. An optical filter with a centre wavelength of 405 nm and bandpass of 10 nm was placed at the input to the fibre optic to match the absorbance wavelength of 4-nitrophenol at pH 7.5 (absorbance coefficient, ε_{405}=1700 m^2 mol^{-1}). To establish which of the image pixels were to be used as 'reference pixels' and which to use as 'sample pixels' snapshots with the capillaries filled with water were compared with those with the capillaries containing a 1 mM solution of p-

nitrophenol. The average pathlength is $\pi d/4 = 79$ μm; this corresponds to an absorbance of 0.13 AU for this solution. The pixels that showed a reduction in signal in the presence of the p-nitrophenol solution that corresponded to an absorbance of <0.02 were used as reference pixels, those with > 0.1 were used as sample pixels.

Capillary electrophoresis and data processing

A series of parallel capillary electrophoresis experiments were carried out using a pH 7.5, sodium phosphate buffer (15 mM sodium) on dilutions of a p-nitrophenol solution made up in buffer. Injections were performed inserting the inlet end of all the capillaries into the sample solution that was held at 2 cm above the level of the buffer in the outlet vial for 15 s. The volume injected into each capillary by siphoning should be approximately 16 nL. A voltage of +5 kV was applied to the inlet vial and the electrophoresis was carried out; electroosmotic flow ensured that the analyte moved towards the grounded cathode at the outlet. The snapshot data was accumulated in the computer RAM during the experiment and saved to disk at the end of a run.

The raw data collected during an experiment was first corrected for fixed pattern noise as described in detail in "A charge coupled device array detector for single-wavelength and multi-wavelength ultraviolet absorbance in capillary electrophoresis", Bergstrom and Goodall, Pokric and Allinson, Anal. Chem. 1999, 71, 4376-4384, and then processed to produce a set of electropherograms, one for each capillary. This ensures that high spatial resolution is maintained, limited only by the dimensions of the effective pixels used, 106 μm in this case.

Figure 9 shows the four electropherograms obtained using a sample solution of 100 μM. It is clear from the dips in the base line at times corresponding to peaks in adjacent capillaries that there is cross talk between capillaries. This is mainly caused by some light passing through the sample being used as part of the reference, and to a lesser extent there is some light that after passing through the sample in one capillary lands on a sample pixel of an adjacent capillary. The extent of both of these effects has been measured by filling each capillary in turn with the sample solution. It has been assumed that the level of cross talk scales linearly with absorbance and the correction based on the measurement at this single sample concentration has been applied to all subsequent experiments. The appropriate correction is readily made as is demonstrated by Figure 10.

Noise performance

Figure 11 shows the four electropherograms obtained by injecting a solution of 1 μM 4-nitrophenol. The peak heights in Figure 11 correspond to a concentration of ~0.6 μM, the limit of detection is calculated to be 0.22 μM based on three times the RMS baseline noise of 9.4 μAU (1 s risetime). The theoretical shot noise limited baseline noise level was calculated by generating simulated data sets based on the average exposure patterns and levels found in the experiment and assuming a pixel full well capacity of 5×10^5 electrons. These generated snapshot data sets were processed identically to the experimental data sets; the RMS shot noise level was found to be 9.0 μAU. This is very close to the observed value and indicates that the detector is shot noise limited.

The CCD used in this experiment is large enough to accommodate over 30 capillaries aligned as the four are here. The sensitivity of detection for each

of these capillaries would be the same as found in this study (i.e. ~ 9 μAU RMS noise with a rise-time of 1 s). Alternatively over 120 capillaries could be accommodated if they were aligned parallel to the short dimension of the CCD; this would result in a reduction in the signal integration time for each capillary by a factor of four and therefore an increase in noise level by a factor of $\sqrt{4} = 2$. Capillary electrophoresis with arrays of capillaries obviously has application for high throughput analysis as the number of separations that can be carried out simultaneously simply scales with the size of the array. However, if the same sample is simultaneously injected into all the capillaries in the array, as done in this experiment, it is a means to increase sample loading and therefore dynamic range and concentration sensitivity. If all of the electropherograms are combined by taking the arithmetic average after compensation for the slight differences in analyte velocities between capillaries, then an increase in signal to noise of $\sqrt{N}$ is achieved, where N is the number of capillaries. Splitting a large sample load into many capillaries reduces the problems associated with overloading which include poor peak shape induced by electromigration dispersion and large injection lengths, and nonlinear detector response at high absorbances of highly abundant components. Figure 12 shows the result of averaging the four electropherograms of Figure 11. The RMS baseline noise level is 4.7 μAU, a reduction by a factor of 2 of the individual traces, this is the improvement expected for 4 capillaries.

The noise performance measured by combining the signal from four capillaries is still at the shot noise limit and is better than the performance achieved previously in Bergstrom et al above, where it was suggested that fluctuations in the spatial distribution of the lamp discharge, which is imaged on the fibre bundle input, would result in fluctuations in the spatial

distribution of the CCD illumination. This would result in a noise level that is in excess of the shot noise because separate areas of the CCD monitor the signal and reference levels. Using a single optical fibre instead of the fibre bundle used previously should to a large degree scramble any spatial inhomogeneities of the lamp discharge. Scaling up the experiment to 30 parallel capillaries should give a combined RMS baseline noise level of 1.7 μAU, corresponding to an on column p-nitrophenol concentration LOD of 40 nM.

When the analyte concentration is low relative to the concentration of background electrolyte then it should only be the length of the injection plug and analyte diffusion that contribute significantly to the measured peak width, assuming that the spatial resolution of the detector is high. The peak variance caused by analyte diffusion can be calculated, $\sigma^2=2Dt = 1.2 \times 10^{-6}$ m^2, where D is the diffusion coefficient (8.1×10^{-10} m^2 s^{-1} for p-nitrophenol) and t is time. The contribution to the peak variance from the length of the plug of sample injected is given by $l_i^2/12 = 0.4 \times 10^{-6}$ m^2 (where l_i is the injection length of 2.0 mm). These two contributions give a total standard deviation, σ, of 1.3 mm; dividing by the analyte velocity gives $\sigma = 3.2$ s in units of time. This compares well with a standard deviation of 3.1 s measured by fitting a Gaussian function to the peak in Figure 12. This is experimental evidence that the detection method of imaging a 26.6 mm capillary section and of subsequently combining parallel electropherograms does not degrade separation efficiency.

Example 2

Table showing minimum i.d. for vessel with circular outer and inner cross sections to obtain spatial separation between core and wall beams. Values are shown for water and methanol (RI = 1.33) and acetonitrile (RI = 1.39).

	minimum i.d./o.d.	
solvent in vessel core	water or methanol	acetonitrile
d /o.d.		
1/2	1/2	1/3
1/1	1/3	1/4
2/1	1/4	1/6

d is the separation between vessel and array detector; o.d. = outer diameter; i.d. = inner diameter.

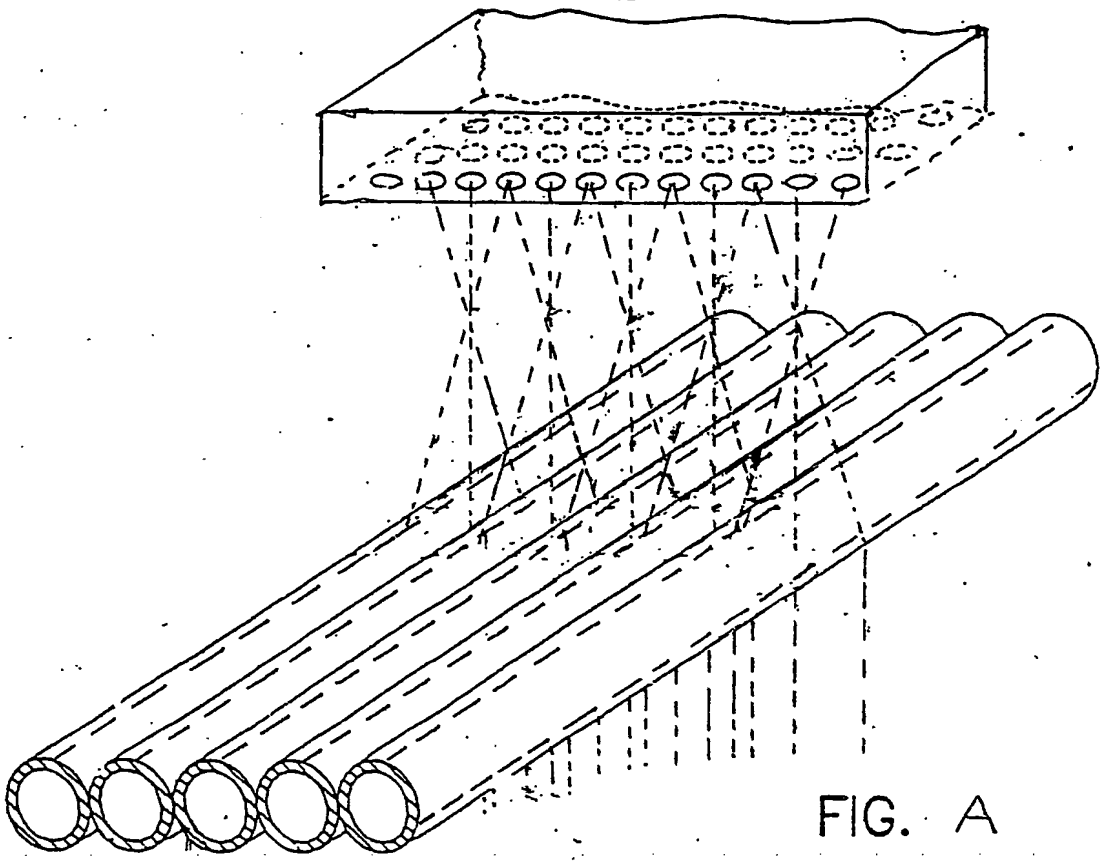
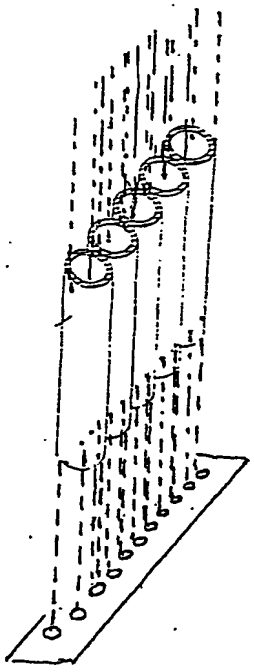


FIG. A



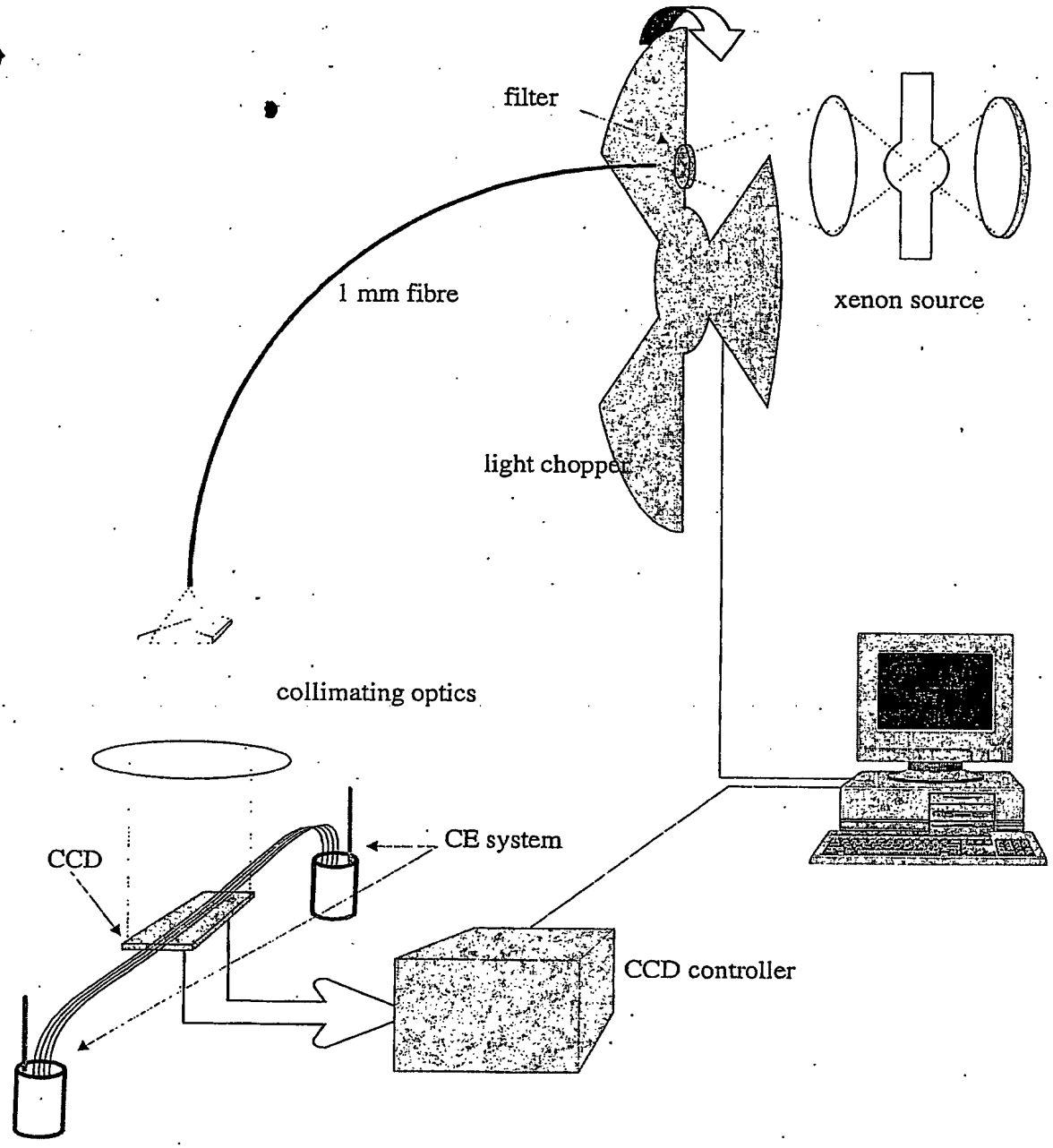


Figure 1. Schematic diagram of experimental apparatus for parallel capillary absorbance detection.

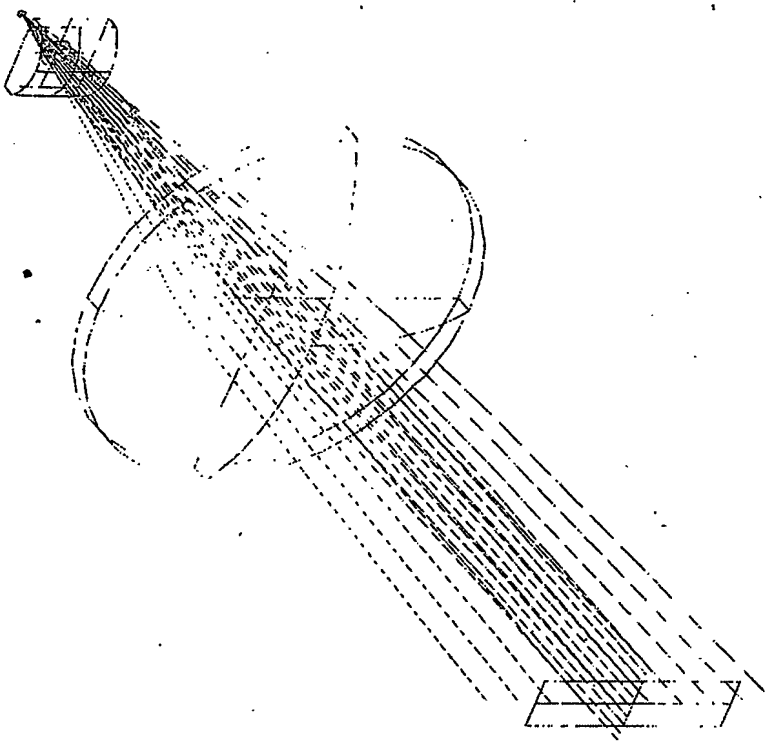


Figure 2. Collimated illumination of rectangular CCD area, (26.6 x 6.7 mm) using light output from a 1 mm diameter fused-silica optical fibre (N.A. = 0.22) using a cylindrical and spherical fused-silica lens elements.

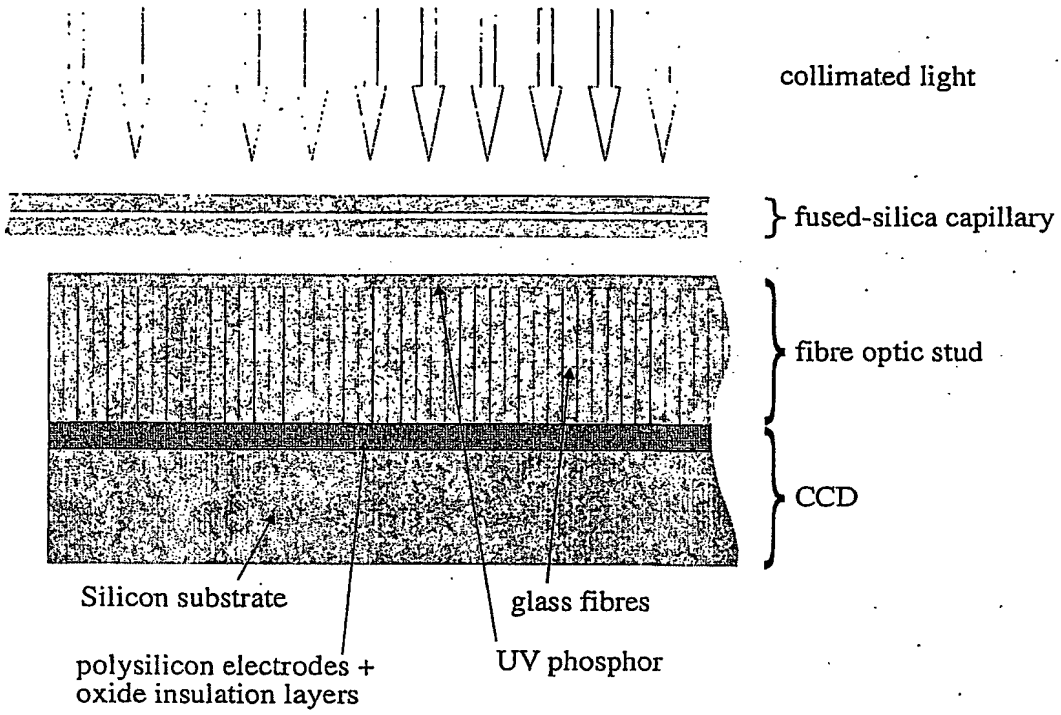


Figure 3. Detail of CCD with fibre optic stud and imaging of capillaries.

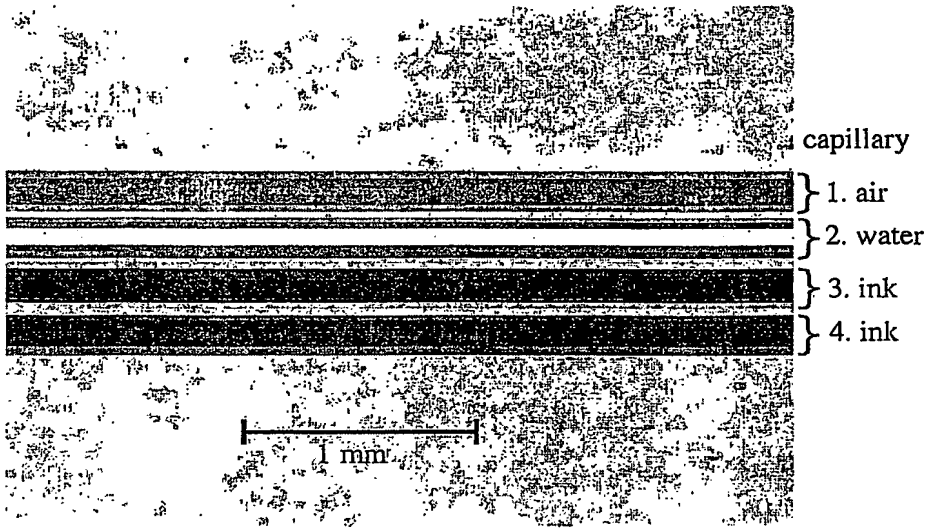


Figure 4. Part of one CCD snapshot showing ~3 mm of 4 capillaries (100 μm i.d., 194 μm o.d.); the total area imaged is 6.7 × 26.6 mm. The contents of the capillaries are, 1. air, 2. water, 3 & 4 ink solution.

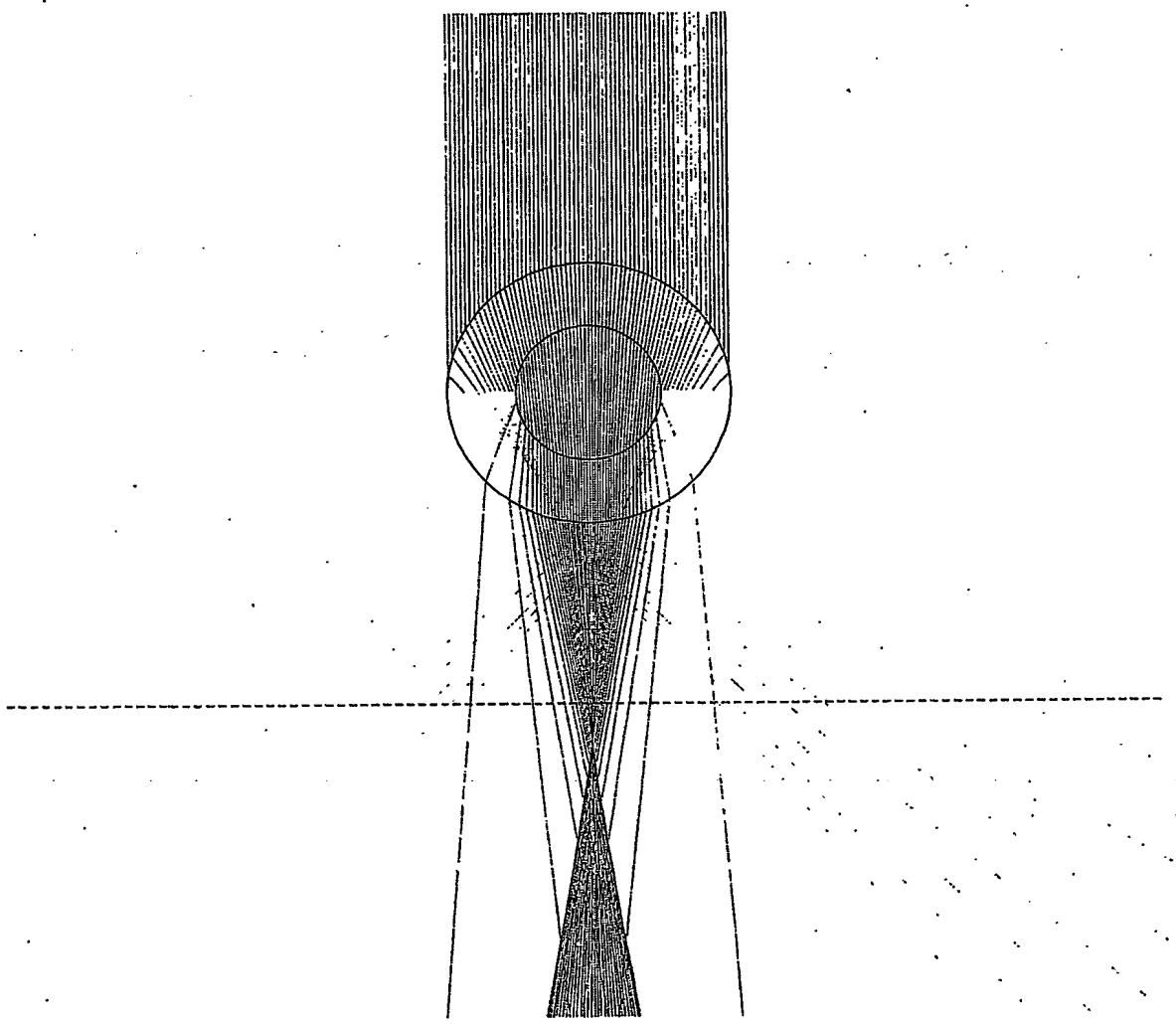


Figure 5. Ray tracing diagram show light path through a water-filled fused-silica capillary, 100 μm i.d., 194 μm o.d. The dark rays represent the light passing through the water at the capillary centre and the light rays show the light that passes only through the capillary walls. The dashed line shows the approximate position of the fibre optic stud surface.

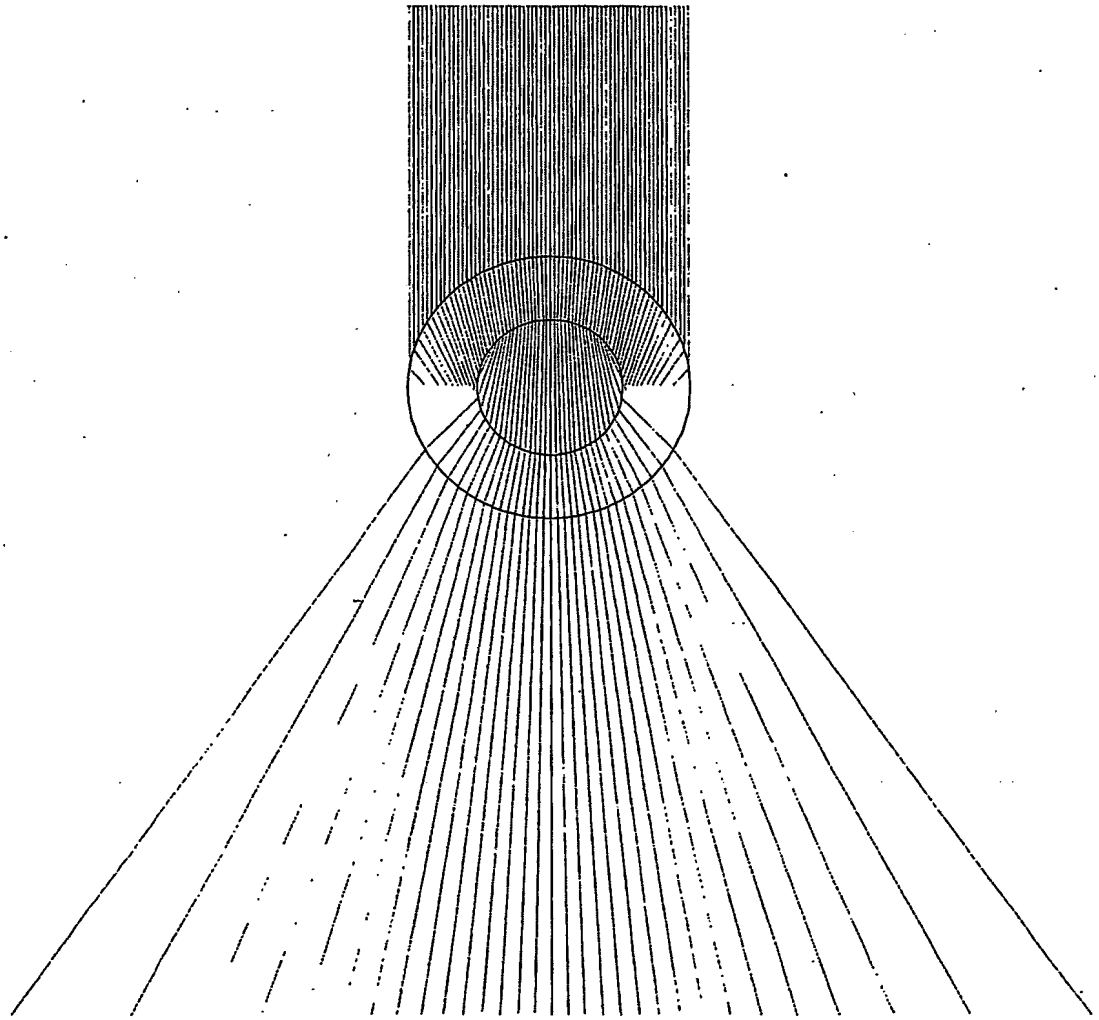


Figure 6. Air filled capillary, 100 μm i.d., 194 μm o.d.

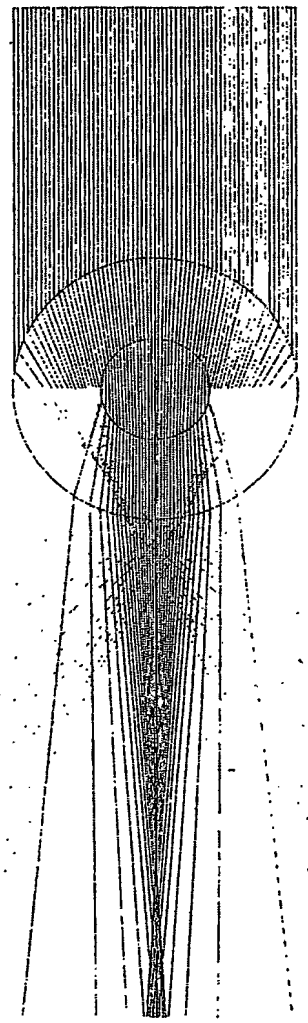


Figure 7. Water filled capillary, 75 µm i.d., 194 µm o.d.

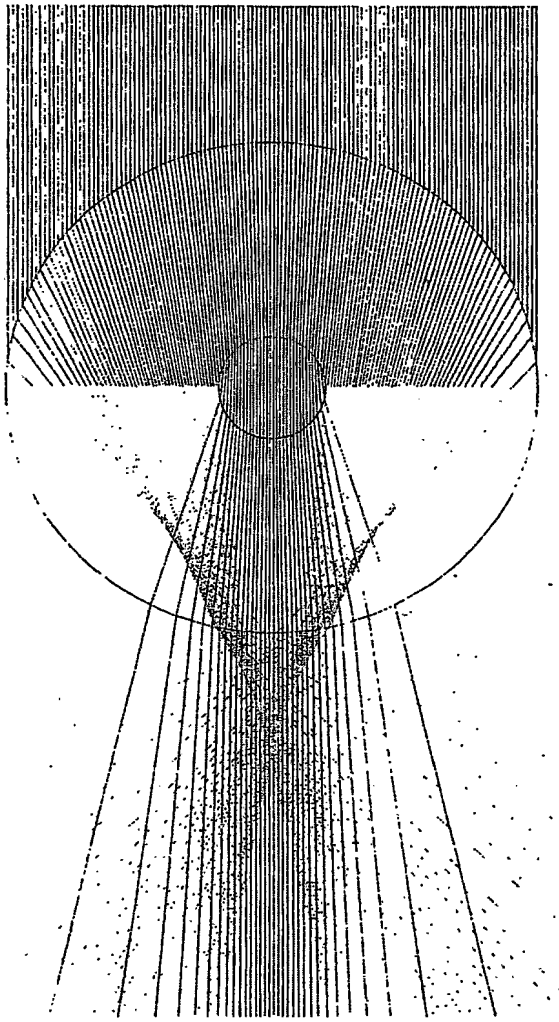


Figure 8. Water filled capillary, 75 μm i.d., 364 μm o.d.

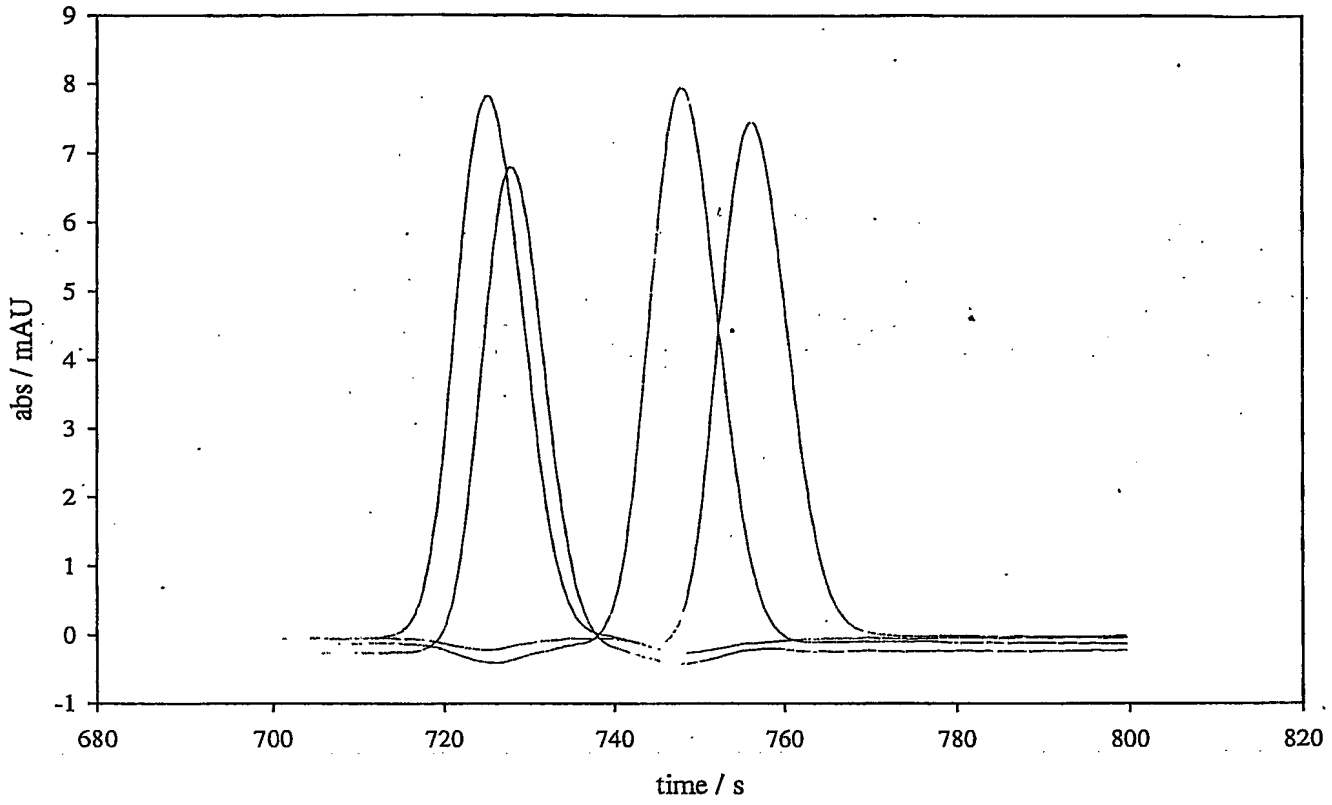


Figure 9. Electropherograms of ~16 nL 100 μM p-nitrophenol injected into each of four parallel 100 μm i.d. capillaries. Capillary length: 500 mm total, 300 to the detector. Separation voltage: 5000 V. Buffer: sodium phosphate pH 7.5 (15 mM sodium).

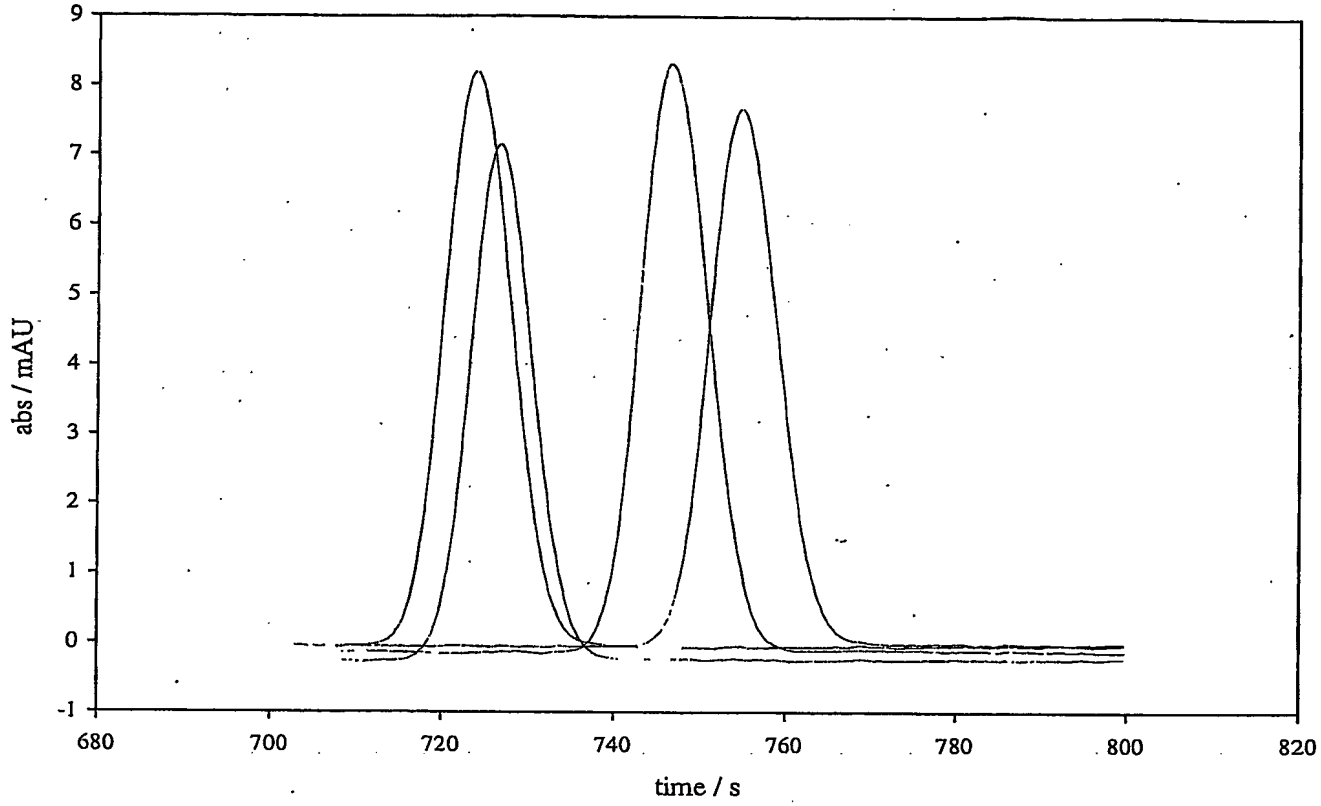


Figure 10. Electropherograms of 100 μM p-nitrophenol after correction for cross-talk between capillaries.

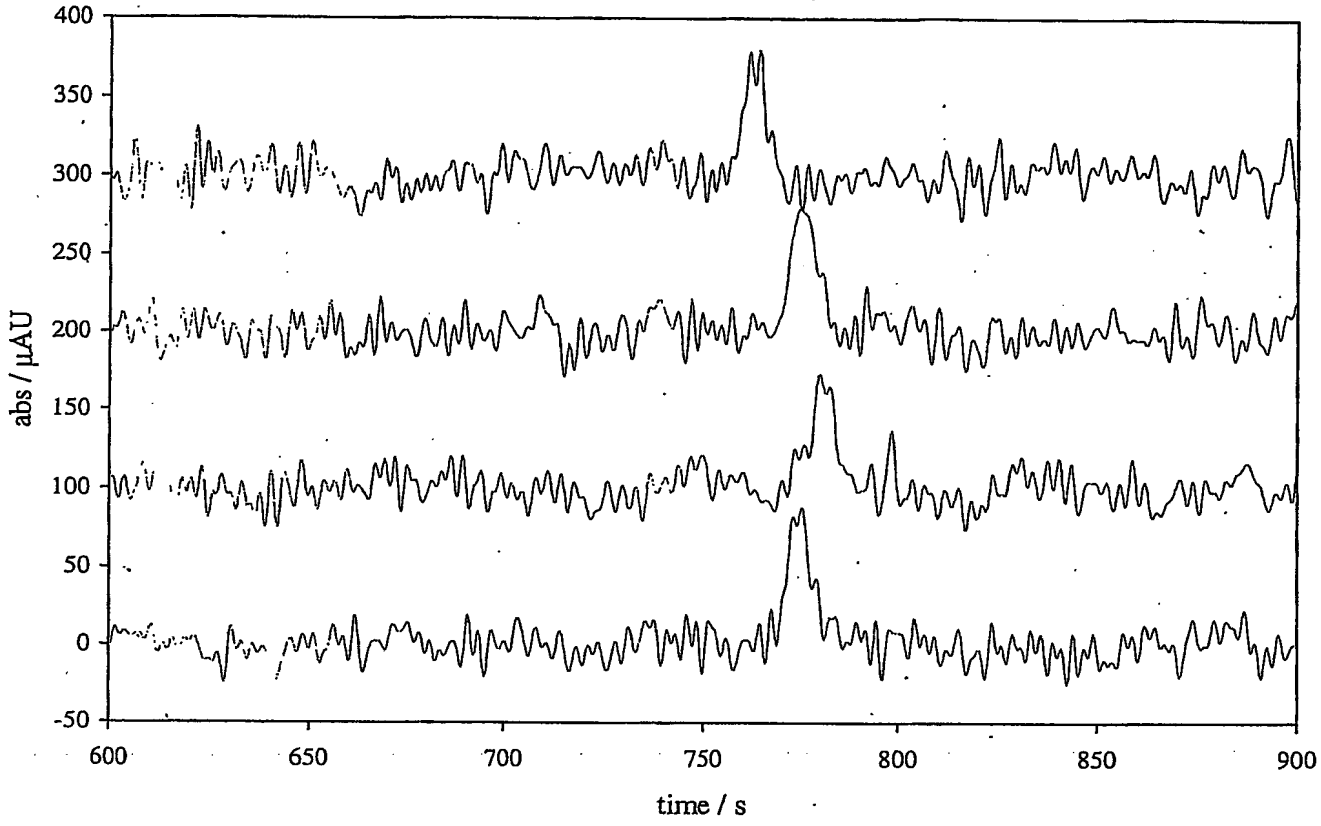


Figure 11 Electropherograms of ~16 nL 1 µM p-nitrophenol injected into each capillary.

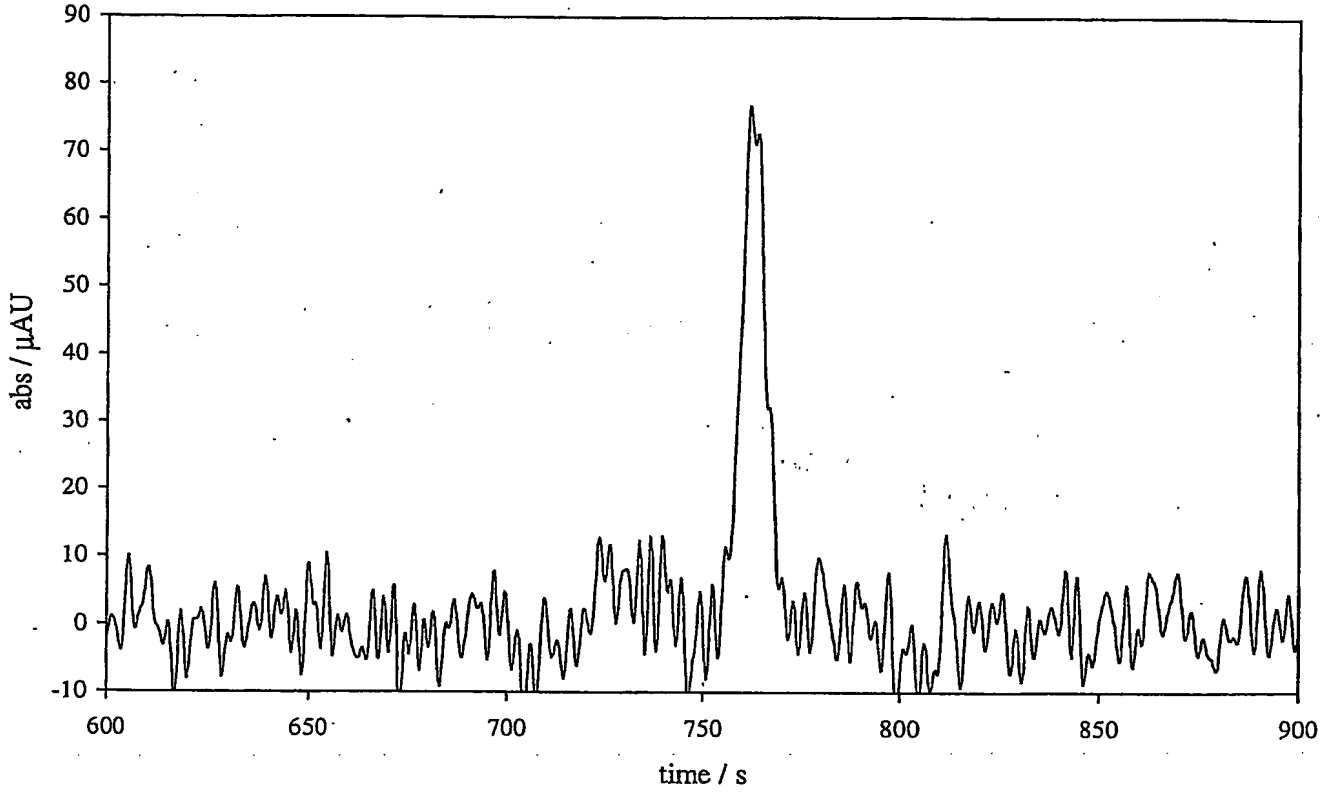


Figure 12 Electropherogram generated by taking the average of the four traces shown in figure 12.